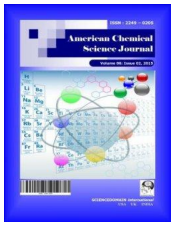

American Chemical Science Journal
9(2): 1-19, 2015, Article no.ACSJ.20257
ISSN: 2249-0205

SCIENCEDOMAIN *international*
www.sciencedomain.org

Comparison of Analytical Techniques in the Characterization of Complex Compounds

F. S. Nworie[1*], F. I. Nwabue[1] and W. J. O. Oti[1]

[1]Department of Industrial Chemistry, Ebonyi State University, PMB 053, Abakaliki, Ebonyi State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author WJOO designed the study. Author FSN performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors FIN and WJOO managed the analyses of the study. Author FSN managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACSJ/2015/20257
Editor(s):
(1) Georgiy B. Shul'pin, Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow, Russia.
Reviewers:
(1) Anonymous, India.
(2) P. Krishnamoorthy, Madras University, India.
Complete Peer review History: http://sciencedomain.org/review-history/11194

Review Article

Received 18th July 2015
Accepted 5th August 2015
Published 31st August 2015

ABSTRACT

The methods of characterization of complex compounds with the intent of their applicability in identification procedures have been critically appraised. In this work, the principle, sensitivity, selectivity and applicability of the different analytical techniques have been systematically presented. The analytical techniques were compared based on the strength of information provided for, which is germane for complex compound identification.

Keywords: Complex compounds; analytical techniques; characterization; comparison.

1. INTRODUCTION

Complex compounds differ between each other because of the diverse range of elements, number of atoms and characteristics of the derivatives. As the number of and diversity of atoms, conjugations, bonds, molecular conformations and or configurations increases, the complexity and of course the analytical method to employ for reliable result also

**Corresponding author: E-mail: nworie.felix@gmail.com;*

increases. Thieme and Muller [1] noted that an analytical process involves getting of information. This generation of information can be employed for the purpose of identification, structural elucidation and or characterization. However, the potentialities of the processes are not the same and have continued to change due to innovation.

Many methods abound for characterizing complexes starting from measurement of the physical characteristics such as melting point, boiling point, colour, conductivity, crystal shapes, elemental content, molar mass to spectroscopic or structural methods such as UV-vis absorption spectroscopy, IR absorption spectroscopy, Raman spectroscopy, Nuclear magnetic resonance, magnetic circular dichroism, cyclic voltammetry and x-ray methods. In all these methods, it was noted [2] that molecular spectroscopy (mass spectroscopy) which provides more information is preferred for identification and as an analytical technique all things being equal. Studies [2] also observed that even though emission spectral analyses generate a lot of information, it is not applicable for molecular analysis as it has numerous identification problems. Studies [3] have observed that larger number of identification points which is an expression of higher selectivity in analysis is achieved by the use of proper methods of analysis. This assertion was based on statistical data and articles related to identification prove [3]. Studies have shown that mass spectrometry and chromatography mass spectrometry posses the highest potential for qualitative identification and determination of complex compounds in complex matrices [2]. The technique is better than other spectral methods in the area of selectivity, generation possibility of molecular formula or mass, sensitivity and its ability to be combined with chromatography. The essence of gas or liquid chromatography incorporated as an inlet device is to separate complex mixtures of chemical compounds prior to their detection and recognition. Raftery [4] noted that analysis time and measuring limits have been drastically reduced with the evolution in NMR. Studies [5] have shown that the solution of stereochemical problems is of paramount importance in NMR technique. He noted that NMR is indispensable for structural elucidation and spectral interpretation of pure chemical compounds.

The spectral values are highly sensitive to variations in molecular conformations and configurations and hence the essence of molecular recognition in complex characterization. In complex characterization by the use of NMR, two recent approaches have been advanced; the computer spectral simulation and experimental NMR spectra. In the first, spectra from hypothetical or theoretical structures are compared with those spectra recorded for analytes whereas in the second, the experimental is compared with NMR spectra of reference database. Nevertheless, studies [5] noted that the application of NMR in qualitative analysis is limited because of relatively low sensitivity and low identification power in relation to individual components of complex matrices.

The elucidation and identification of compounds by means of spectral interpretation with the aid of reference tables containing wave number of absorption bands of different functional groups and specific band absorption remains the task of classical IR spectroscopy [6]. When analyzing simple compounds with IR spectroscopy, it has been noted [7] that the number, energy and intensity of the IR transitions are directly related to the geometry of the compound and shows which atom is attached to the other. A major limitation of this analytical technique is that for complex compounds in which the organic moieties is large, the IR becomes very difficult to interpret. This may be as a result of vibrations for heavier elements which occur outside the frequency window of most commercial instruments. Other limitations of this method according to studies [8] include relatively low sensitivity and low compatibility of the instrument IR detector with gas and liquid chromatography. A complementary method of analysis to IR spectroscopy is Raman spectroscopy. The method applied in both is the same as both probes vibrations in a compound but the selection rules is different. A number of investigators [7,8] have noted that consideration of the peaks present or absent in the IR and Raman spectra of a compound can help in the determination of the geometry. However, Raman spectroscopy has an edge over IR spectroscopy because the former depending on the design of the instrument can scan to very low frequencies (-100 cm^{-1}) which is too low for the later absorption.

The work of Ferraro, Nakamoto and Brown [9] have shown that the combination of IR and Raman spectroscopy known as vibrational spectroscopy gives complementary information for the exact functional group present in a compound. The advent of resonance Raman, a

variant technique can be used to assign vibrations and identify ligands. Due to technological advancement studies [8] have revealed that the modern state of the IR technique using Fourier transformed – IR instruments is characterized by widespread application of electronic libraries of IR spectra, minimal or no sample preparation, Fast determination and reduced cost.

The application of UV-vis spectroscopy rarely leads to unambiguous identification of individual compounds as the spectra characterize chromophores rather than individual compounds. The method according to Ebsworth et al. [7], however provides the number, energies and intensities of a metal compounds absorption bands in the UV-vis and near IR and can be applied in the determination of the type of atom bound to a metal and the geometry about the metal.

X-ray diffraction or crystallography has been noted as a useful and invaluable characterization method for the identification of crystalline phases and structural determination of inorganic and organic solids [10]. Studies [11] have shown that x-ray crystallography gives detailed atomic positions from precise measurement of the intensity and angles at which x-ray beam diffracts off a crystal. Consequently, it was observed [12] that x-ray diffraction remains the most common method for the examination of molecular structure and is dependent on the scattering of x-rays by the electron density of atoms and molecules. Kapoor and Batra [13] have shown that powder x-ray diffraction can be used to obtain the dimensions of the unit cell for identification. Consequently powder x-ray diffraction is used to determine the lattice parameters, to identify and determine whether a sample is a mixture of more than one crystalline substance, to detect change in lattice structure, to calculate thermodynamic parameters, to show the degree of crystalline substance, to detect change in lattice structure, to calculate thermodynamic parameters, to show the degree of crystallinity and to determine the molecular structure of the material.

Mass spectroscopy has been applied for the determination of molar mass of compounds and in combination with elemental analysis helps in the determination of the chemical formula of the compounds. The complex fragmentation patterns of inorganic compounds can be used for structural elucidation [7]. The development of Electrospray Mass Spectrometry (ESMS) by Fenn and co-workers in mid 1980's exploited the analysis of high molecular weight materials (protein and polymer), fragile materials and most recently co-ordination complexes [14]. Traeger and Cotton [15] noted that the process involves the transfer of solution ions of the analyte to the gas phase through a gentle ionization process. This ionization process gives molecular ions which uses an established technique such as collision –induced dissociation (CID) in the fragmentation process to yield structural information. The advantages of electrospray ionization are that the sample is introduced as a dilute solution (which encourages direct analysis of reaction mixture, gentle nature of the ionization process, little sample in picomolar concentration for analysis which is accessible under ideal conditions, spectra are simple to analyze, the system can be coupled with liquid chromatography separation technique and there is absence of sample volatility [16]. Time of flight mass spectrometry (TOFMS) is used to analyze large molecules due to its broad range in mass. In TOFMS, ions are introduced to the mass spectrometer in well defined time pulses. The detection of ion by TOF instruments is basically different from other mass spectrometers as the process is temporal unlike others that are spatial. It has been noted [17] that the entire mass spectrum of TOFMS is recorded on a microsecond time scale for each ionization event and as such many spectra can be recorded in a very short period, the instrumentation is simpler when compared to scanning mass spectrometers, more sensitive than scanning mass analyzers and unlimited mass range for ion detection. However, the major limitation of TOFMS is that it has poor mass resolution when compared with other types of mass spectrometers.

Circular Dichroism (CD) spectroscopy has been empirically used in inorganic chemistry and in particular co-ordination chemistry for analytical information and structural elucidation of transition metal complexes with enantiometric ligands. Studies [18] have shown that not only is CD used to measure the degree to which transition metal complexes are structurally analyzed; it offers a versatile tool for abstracting important information on the interaction of metal ions with large biologically active molecules such as proteins and DNA.

The effects that contribute to the overall CD signal measured for a co-ordination compound

were listed by some investigators [18,19]. These effects include chiral arrangement of chelate rings around the metal centre (configuration effect), the equivalence in co-ordination compounds of the chiral carbon atoms in organic compounds (inherent dissymmetry), the ligands is enantiometrically pure if its chirality is induced into electronic transitions involving mainly the metal centre (vicinal effect), the ligand preferentially assumes a chiral conformation (conformational effect) and presence of chiral distortions in the co-ordination compound (symmetry distortion effect).

Circular dichroism spectroscopy is importantly used in speciation studies. Pessoa et al. [18] noted that when a metal ion and a chiral compound are dissolved in an aqueous solution and a circular dichroism spectrum is in the visible range as a result of d-d transitions, there is evidence of complexation and if there is absence of chiral ligand, bound to the metal ion, CD is zero. The importance of circular dichroism spectroscopy is the application of its spectra in speciation studies. This is applied for identifying complex species, establishing equilibrium models, in detecting interactions of metal ions with biological molecules, to follow reactions of co-ordinated ligands and for the calculation of stability constants of complexes.

Magnetic circular dichroism (MCD) a hybrid technique which is based on the ability of all matter to rotate the plane of polarized light in the presence of a magnetic field combines both spectroscopic and magnetic properties to measure the magnetic and electronic properties of a compound. The method is importantly most useful for paramagnetic compounds and can be used in a mixture if transition arising from the various species can be identified. The major limitation to this however, is that the process is usually performed at low temperature (<77K) and the process uses strain free glasses therefore limiting its use [7].

Electron paramagnetic Resonance (EPR) or Electron spin Resonance (ESR) is used for the determination of the number of unpaired electrons, geometry about the metal centre and the ligands surrounding the metal for a paramagnetic compound. The NMR though related to EPR and sometimes is the same is usually used for diamagnetic compounds. A new technique that combines the two methods is Electron Nuclear Double Resonance spectroscopy (ENDOR) [2,7].

Thermogravimetry is used for the determination of a materials thermal stability and fraction of the volatile components as a specimen is heated and the weight change is monitored. In other words, it involves a group of technique in which the physical and or chemical properties of a material is recorded against time or temperature variation when the sample temperature follows a program that is pre-established. In determining the changes in mass of a complex due to water loss/decomposition either of the two thermogravimetric methods is used. Thermogravimetric analysis of complexes are used to abstract information about the thermal stability of complexes, decide whether water molecule if present in a complex is in the inner or outer co-ordination sphere and suggest a general scheme for the thermal degradation of a complex [20,21]. The derivative thermogravimetry (DTG) which determines mass change due to dehydration, decomposition or oxidation and differential scanning calorimetry (DSC) or differential thermal analysis (DTA) which deals with thermal effects such as phase transitions, decompositions and oxidations. Thermal analysis methods have been used for the evaluation of materials and product such as determination of thermal stability, degradation degree, quality control thermal life time prediction, thermo oxidative stability and assessment of environmental impact on the materials as products [22,23].

In some cases, many characterization methods are applied on a single sample forming multilevel chromatographic sequence. In such a situation, the sample is passed through high performance liquid chromatography (HPLC) for fractionation, and then the molecular size and weight are analyzed using gel permeation chromatography (GPC). The first spectroscopic method applied in such a situation is a UV detector which shows the characteristic bonds present and then FT-IR for analysis of composition, NMR for analysis of branching, DLS (dynamic light scattering) for determination of absolute molecular weight and matrix assisted laser desorption/ionization spectroscopy (MALDI) for analysis of chemical head groups. This is illustrated in Fig. 1.

In complex characterization and analysis, chromatography is a separation method for measuring molecular weight distributions while DLS (dynamic light scattering) measures or monitors diffusive processes in soft matter since macromolecules undergo diffusive Brownian motion in solution as noticed in time dependent fluctuation of the scattering intensity [21].

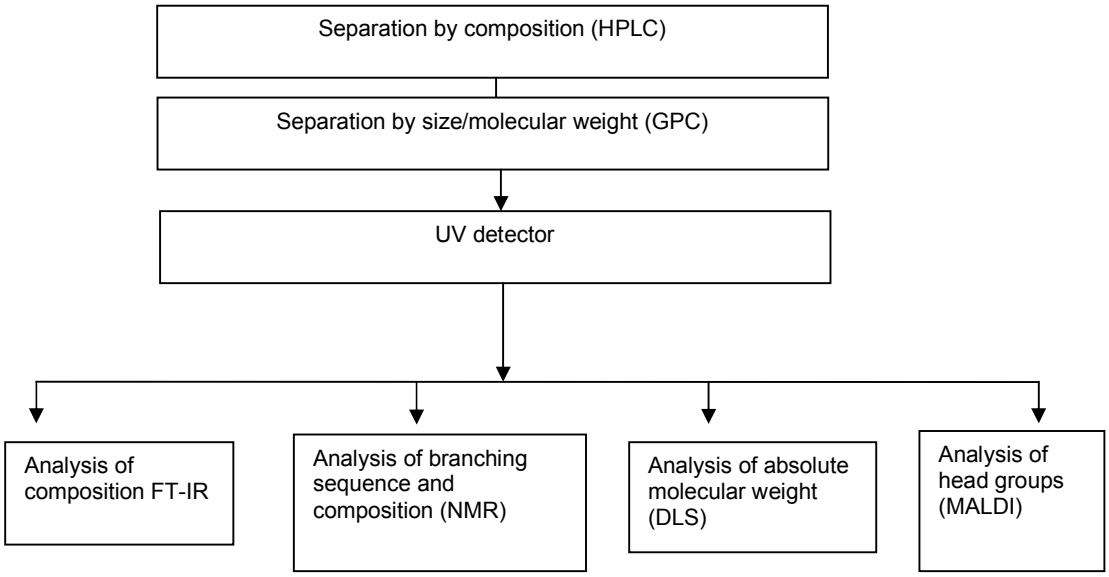

Fig 1. Multilevel characterization sequence

2. OVERVIEW OF ANALYTICAL METHODS IN COMPLEX COMPOUNDS CHARACTERIZATION

Analytical methods deal with the determination of the chemical composition of samples of substances through information about the identity of molecular species or atomic or functional groups or relative amount of the components. Basically, there are two types of analytical methods, classical and instrumental. Classical methods of analysis are the oldest analytical procedure which involves separating the components of interest in a given sample by extraction, distillation or precipitation. Consequently, colour development follows which helps in analyte identification and characterization by boiling and melting points, solubilities, odours, refractive indexes and or optical activities. To quantify the analyte, gravimetric or titrimetric analysis is performed to determine the mass of the analyte or compound formed from the analyte and to determine the volume or mass of a standard reagent needed to react completely with the analyte respectively. Even though these classical methods are still in use in some laboratories, their general applicability is decreasing as instrumental analysis has come into full action early in the twentieth century [2].

Instrumental methods involve the qualitative and quantitative determination or analysis of a variety of inorganic, organic and biochemical analytes by employing conductivity, electrode potential, light absorption or emission, mass-to-charge ratio, fluorescence, chromatography, and electrophoretic techniques [24a].

Table 2.1 shows the instrumental methods, chemical and physical properties and method of analyte stimulation.

Studies [24a] have noted that instrument for chemical analysis transforms information in the chemical and physical compositions of analyte to information manipulatable and interpretable by human. The retrieval of the information as desired from the analyte comes in the form of electromagnetic, electrical, mechanical or nuclear energy. The instruments for chemical characterization contain some components which are listed in Table 2.2.

In analysis involving instrumentation, the detectors and sensors are different. Detectors are mechanical, chemical or electrical devices which identifies, indicates or record a change in one of the environmental variables such as temperature, pressure, molecules, nuclear radiation and electrical charge. Sensors are class of analytical devices that monitors the particular chemical species (speciation) continuously and reversibly. This means that the sensor contains substances that respond to physical or chemical characteristics of an analyte specifically. UV detector is an example of detector whereas glass electrode is an example of sensor.

Table 2.1. Instrumental methods, chemical and physical properties and method of analyte stimulation, Skoog et al. [24a]

Instrumental methods	Characteristic properties	Method of analyte stimulation
Emission spectroscopy (X-ray, UV-visible electron)	Emission of radiation	Interaction with electromagnetic radiation
Spectrophotometry and photometry, NMR, Electron spin resonace spectroscopy, IR, UV, X-ray	Absorption of radiation	Interaction with electromagnetic radiation
Raman spectroscopy, nephelometry and turbidimetry	Scattering of radiation	Changes in electromagnetic radiation
Refractometry, inteferometry	Refraction of radiation	Changes in electromagnetic radiation
X-ray and electron diffraction methods	Diffraction of radiation	Changes in electromagnetic radiation
Polarimetry, circular dichroism	Rotation of radiation	Changes in electromagnetic radiation
Potentiometry	Electrical potential	Electrical properties
Coulometry	Electrical charge	Electrical properties
Amperometry, polarography	Electrical current	Electrical properties
Conductometry	Electrical resistance	Electrical properties
Mass spectrometry	Mass –to-charge ratio	Mechanical property
Thermal gravimetry and titrimetry (DSC, DTA) and thermal conductometric methods	Thermal characteristics	Mechanical property
Gravimetry	Mass	Mechanical property
Radioactivity	Activation and isotope dilution methods	Nuclear properties

Table 2.2. Components of analytical instruments and their example, **Skoog et al. [24a]**.

Instrument components	Examples	Functions
Energy source (stimulus)	Tungsten lamp, flame, Direct current source, glass electrode, sunlight, x-ray tube	Elicits response from analyte as governed by fundamental laws of chemistry and physics
Input transducer	Photocell, photomultiplier tube, electrodes, eye, photographic film, glass calomel electrodes	Converts information from nonelectrical domains to information in electrical domains and vice versa
Data domain of transduced information	Electrical current, electrical potential optic nerve signal and latent image	Shows how information flow as governed by rules of data domain transformation
Information processor	Meter scale, amplifier,digitizer, chemical developer, brain	Processes information from nonelectrical domains to electrical domains
Read out	Current meter, chart recorder, black images of film, visual colour response	Converts information from electrical domain to a domain understandable to human observer e.g. graphic output or alphanumeric output

Studies [24a] have shown that in selecting an analytical problem, it is germane to clearly define the nature of the analytical problem. Consequently, the problem of accuracy, concentration of analyte, mass of sample available, physical and chemical characteristics of the sample matrix, the number of sample to analyze and the quantity or components of the sample that will cause interference. Therefore, in consideration of the above factors, the

quantitative performance criteria of the instrument will substantially determine which method is suitable for an analytical problem.

In selecting the analytical methods, the following criteria are always considered (1) precision (relative standard deviation, coefficient of variation, variance (2) Bias (Absolute systematic error and relative systematic error) (3) sensitivity (analytical sensitivity and calibration sensitivity) (4) Detection limit (blank plus three times standard deviation of a blank) (5) concentration range (limit of quantification) (6) selectivity (coefficient of selectivity).

3. SPECTROMETRIC METHOD OF ANALYSIS

Spectrometric method of analysis otherwise regarded as spectroscopic methods are large group of analytical method of characterizing substances based on molecular and atomic spectroscopy (the interaction of radiation with matter). Skoog et al. [24a] noted that spectrometry and its method involves the measurement of the intensity of radiation (electromagnetic gamma rays, x-rays, ultraviolet, microwave and radio frequency radiations) with an electronic device such as photoelectric transducer. Some of the spectroscopic method of analysis include ultraviolet visible, infrared, electron spin resonance, electron nuclear double resonance, nuclear magnetic resonance and moss bauer spectroscopy.

Table 3.1 shows the common spectroscopic methods of analysis, the wavelength range, wave number and the type of quantum transition involved. The wavelength is the linear distance between any two successive waves whereas the wave number is the reciprocal of the wavelength in centimeters. In describing wavelength, different units are used based on the spectroscopic method. Take for instance, x-ray and short ultraviolet radiation uses A^0 (10^{-10}m) angstrom unit, visible radiation and ultraviolet radiation uses nanometer, nm (10^{-9}m) whereas infrared radiation uses micrometer, mμ (10-6m).

Molecular absorption spectroscopy deals with the measurement of the transmittance T or absorbance A of solutions Present in transparent cells having a path length of b in cm. The absorption measurements based on visible and ultraviolet radiation is widely used for the quantitative determination of a large variety of organic and inorganic species [24a]. In molecular spectroscopy, the concentration of an absorbing analyte is linearly related to absorbance in what is regarded as the Beer Lambert's law. One of the major advantages of using Beer's law is that it can be applied for mixtures containing more than one kind of absorbing substance in so far that there is no interaction among the various species. However, the major limitations of Beer lamberts law and its application are instrumental deviations and chemical deviations (chemical changes owing to change in concentration).

The absorption of ultraviolet or visible radiation by a molecular or atomic specie is a two step process involving electronic excitation of bonding electrons as indicated by the wavelengths of absorption peaks as correlated with the type of bonds in the species and relaxation processes which may involve the conversion of excitation energy to heat or re-emission of fluorescence or phosphorescence.

Table 3.1. Common spectroscopic methods of analysis, the wavelength range, wave number and the type of quantum transition involved

Spectroscopic method of analysis	Wavelength range	Wave number range cm^{-1}	Type of quantum transition
Infrared absorption and Raman scattering	0.75-300 μm	$1.3x10^4$-$3.3x10^1$	Vibration/rotation of molecules
Gamma ray emission	0.005-1.4A^0	-	Nuclear
X-ray absorption, diffraction, fluorescence and emission	0.1-100A^0	-	Inner electron
Vacuum ultraviolet absorption	10-180 nm	$1x10^6$- $5x10^4$	Bonding electrons
Ultraviolet visible absorption, fluorescence and emission	180-780 nm	$5x10^4$-$1.3x10^4$	Bonding electrons
Microwave absorption	0.75-3.75 mm	13-27	Rotation of molecules
Electron spin resonance	3 cm	0.33	Spin of electrons in a magnetic field
Nuclear magnetic resonance	0.6-10 m	$1.7x10^{-2}$-$1x10^3$	Spin of nuclei in a magnetic field

Consequently, molecular absorption spectroscopy is a valuable tool for the identification of functional groups in a molecule and for the quantitative determination of compounds containing absorbing groups. Electronic transitions can be

(1) Transitions involving ∏, ∆ and n electrons
(2) Transitions involving d and f electrons
(3) Transitions involving charge transfer electrons

Transitions involving n-∏* and ∏- ∏* transitions are the most common in the application of absorption spectroscopy to both organic compounds and inorganic anions as the absorption peaks are brought into an experimentally convenient spectral region of 200 to 700 nm. In these cases unsaturated functional groups to provide the ∏ orbitals are required. n-∏* transitions involve peaks of shorter wavelength (hypsochromic shift) whereas ∏-∏* transitions involve peak of higher wavelength (bathochromic shift).

Most transition metal ions absorb in the visible or ultraviolet region of the spectrum involving the d electrons whereas for the lanthanide and actinide series, absorption results from electronic transitions of 4f and 5f electrons. Many inorganic complexes exhibit charge transfer absorptions with very large molar aborptivities owing to the electron donor –electron acceptor properties exhibited by the species [7].

3.1 Infrared Spectrometry

Infrared spectrometry applies absorption, reflection and emission spectra for the qualitative and quantitative determination of molecular species of all types. The spectra regions employed in the analysis of species include mid infrared which extends between 670 to 400 cm^{-1} (2.5 to 14.9 µm), near infrared region from 4000 to 14, 000 cm^{-1} (0.75 to 2.5 µm) and far infrared region which is primarily used for the structural elucidation of inorganic and metal-organic species based upon measurement of absorption. Far infrared regions are designated as 500-20 cm^{-1} (20-500 µm). It is sensitive to the overall structure of a molecule difficult to detect in mid infrared region.

3.1.1 Mid infrared absorption spectrometry

Structural determination of organic and biochemical species has been the major function of mid-infrared absorption and reflection spectrometry. Infrared absorption measurements take variety of samples ranging from gases, solutions, solvents, liquids, solids, pellets and mulls. Consequently, sample handling is of paramount importance for solid samples are always dispersed in a liquid or another solid matrix and ground until its particle size is very tiny as to prevent scattered radiation. Pelleting has been frequently used in handling solid samples where the solid sample about 1 mg finely powdered is mixed with about 100 mg of dried potassium bromide powder. In a situation whereby solid sample is insoluble in an infrared transparent solvent such as dimethylformamide, chloroform, carbon tetrachloride, benzene, cyclohexane, dioxane and carbon disulphide or are not conveniently pelleted into potassium bromide, the spectra is obtained by dispersing the analyte in mineral oil or fluorinated hydrocarbon mull [24a]. Mull is made by grinding about 2 to 5 mg of finely powered sample in the presence of one or two drops of heavy hydrocarbon oil (nujol).

In qualitative analysis, mid infrared spectroscopy is a two step process which helps in the determination of functional group present in a molecule by examining the group frequency region (radiation from about 3600 cm^{-1} to approximately 1200 cm^{-1}) and comparison of the spectrum of the unknown with the spectra of pure compounds that contain all of the functional groups in the compound of interest.

The spectra of infrared spectroscopy are complex and this enhances the probability of overlap of absorption peaks. There is also non adherence or instrumental deviation to Beer's law, narrowness of the absorption bands and effects of stray radiation. This considerably differentiates infrared spectroscopy from ultraviolet /visible molecular spectroscopy.

However, advantages of FT-IR spectroscopy over dispersive instruments according to studies [25] include high speed of data collection, increased resolution, lower detection limits and greater energy throughput.

The mid infrared region is subdivided into the group frequency region (4000-1300 cm^{-1} or 2.5-8.0 µm) and the fingerprint region (1300-500 cm^{-1} or 8.0-20 µm). According to (Michelle et al. [25], the main absorption bands may be assigned to vibrational modes corresponding to the various functional groups. For instance, NH-OH

(4000-3000 cm^{-1}), C-H stretch region (3000-2800 cm^{-1}), window region (2800-1800 cm^{-1}) and carbonyl region (1800-1500 cm^{-1}).

Single bond and skeletal vibrations of polyatomic systems is as a result of absorption bands in the fingerprint region of the spectrum.

3.2 Raman Spectroscopy

Raman spectroscopy is an analytical method which is applied for the qualitative and quantitative analysis of inorganic, organic and biological systems [24a]. In Raman spectroscopy, the spectra are always acquired by irradiating a sample with a powerful source of laser of visible or near infrared monochromatic radiation and the spectrum of the scattered radiation measured at an angle of 90° using a suitable spectrometer.

Emitted radiation in Raman spectra can be of three types, stokes scattering, anti-stokes scattering and Rayleigh scattering. Rayleigh scattering has a wavelength that is exactly that of the excitation source and is more intense than stokes and anti stokes scattering. In the same way, anti-stoke lines are less intense than stokes line. The polarizability of the molecule, intensity of the source and concentration of the active group in a molecule determines the power or intensity of a normal Raman Peak. In the same way, Raman intensities is directly proportional to the concentration of the active species. For structural determination of molecules in Raman spectroscopy, the depolarization ratio is usually employed. Upon excitation of Raman spectra by plane polarized light radiation, the scattered radiation is found to be polarized to various degrees upon the type of vibration responsible for the scattering. The depolarization ratio is useful in correlating Raman line with modes of vibration and is dependent upon the symmetry of the vibrations responsible for scattering. Raman spectroscopy is a superior technique to infrared spectroscopy for the analysis of inorganic species since aqueous solutions can be used. In the same way, vibrational energies of metal-ligand bonds and estimation of ring size in paraffins are in the range of 100 to 700 cm^{-1} and 700 to 1200 cm^{-1} respectively which is a difficult region of the infrared to study. Consequently, Raman spectra are an invaluable tool for the elucidation of the composition, structure and stability of co-ordination compounds [24a]. In similarity, Raman spectra and infrared spectra are both useful in the identification of specific compounds since they have regions that are useful for functional group detection.

Table 3.2. Group frequencies of organic groups

Bond	Types of compound	Frequency range, cm^{-1}	Intensity
C-H	Alkanes	2850– 2970	Strong
		1340 -1470	Strong
C-H	Alkenes	3010-3095	Medium
	C = C	675-995	Strong
C-H	Alkynes	3300	Strong
	-C Ξ C-		
C-H	Aromatic rings	3010-3100	Medium
		690 – 900	Strong
O-H	Monomeric alcohols hydrogen bonded	3590-3650	Variable
	alcohols, phenols monomeric	3200-3600	Variable may be broad
	carboxylic acids	3500-3650	Medium
	hydrogen bonded carboxylic acid	2500-2700	Broad
N-H	Amines, Amides	3300-3500	Medium
C=C	Alkenes	1610-1680	Variable
C=C	Aromatic rings	1500-1600	Variable
C-N	Amines, Amides	1180-1360	Strong
C=N	Nitriles	2210-2280	Strong
C-O	Aclohols, ethers, esters carboxylic acids	1050-1300	Strong
C=O	Adehydes, Ketones, esters carboxylic acids	1690-1760	Strong
$N0_2$	Nitro compounds	1500-1570	Strong
		1300-1370	Strong

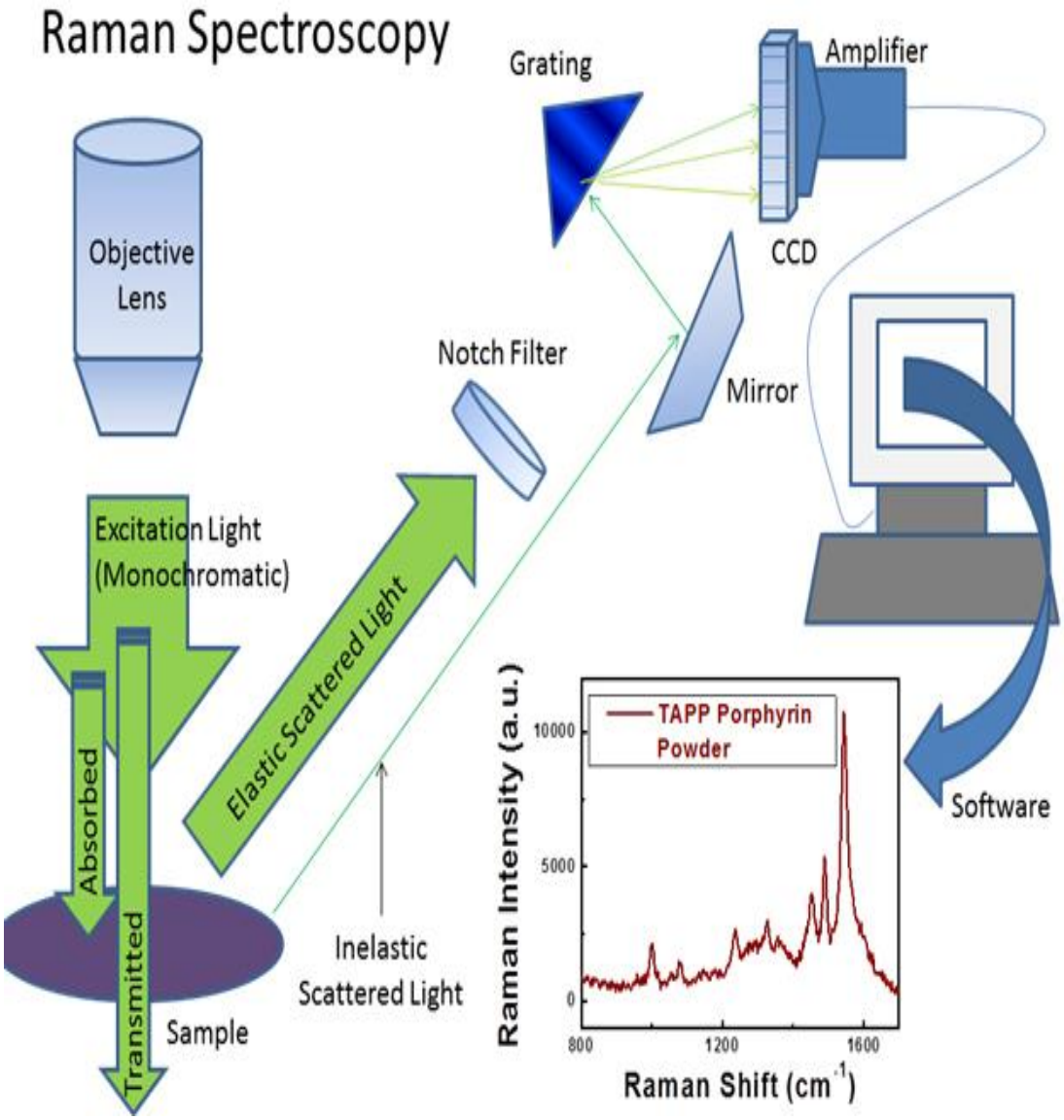

Fig. 2. Diagram of Raman spectrophotometer (Adapted from [24b])

The major limitation of Raman spectroscopy are low efficiency, limitation to visible and near ultra violet regions, susceptibility to interference from fluorescence and its analyte specific nature.

3.2.1 Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is an indispensable and most powerful tool for structure elucidation of chemical species [2]. The operation of NMR is based on the measurement of absorption of electromagnetic radiation in the radio frequency region of about 4 to 900 MHz. In NMR, the absorption process takes place in the nuclei of an atom rather than electron as seen in ultraviolet, visible and infrared absorption. The nuclei are made to develop the energy state required for absorption by subjecting the analyte to an intense magnetic field. The main types of NMR based on the nuclei are ^{1}H, ^{13}C, ^{19}F, ^{31}P, ^{29}Si, $^{17}0$ and ^{15}N. There are also two main types of NMR spectrometers, the continuous wave and Fourier transform or pulsed spectrometers.

The processes in NMR shows that a spinning charged nucleus creates a magnetic field similar

to that produced when electric current flows through a coil of wire. Magnetic moment is produced along the axis of spin which is directly proportional to the angular momentum with a proportionality constant known as magnetogyric ratio. This magnetogyric ratio has values which differ according to the nucleus. Consequently, when a nucleus is exposed to radiation of a suitable frequency, absorption takes place as a result of the slight excess of lower energy state nuclei present in a strong magnetic field. The rate of relaxation of excited nuclei to their lower state must be equal or greater than the rate at which they absorb the radio frequency energy. Non radioactive relaxation processes are of main importance in NMR studies and to reduce saturation, the relaxation should occur as rapidly as possible.

The type of NMR spectra according to studies [24a] is dependent on the kind of instrument, physical state of the sample, type of nucleus involved, purpose of data collection and the environment of the analyte nucleus. Two main types of NMR spectra are known; wideline and high resolution spectra. Wideline spectrum is used for the quantitative determination of isotopes and analysis of the physical environment of the absorbing species. At low magnetic field strength, the bandwith of the source of the lines is large enough so that the fine structure due to the chemical environment is restricted. High resolution spectra is obtained by instruments capable of differentiating between very small frequency differences of 0.0lppm or less and are from several peaks that come from differences in chemical state or environment/nearby electrons and nuclei). The spectra values are sensitive to changes in molecular conformations and configurations.

Studies [2] have shown that classical approach to structure elucidation, spectral interpretation, chemical shifts, spin-spin coupling constants, signal multiplicity are located from reference tables of corresponding measured values. The two types of environmental shifts possible in NMR are chemical shift and spin splitting and are both crucial in structural analysis. Chemical shifts are caused by small magnetic fields generated by electrons as they circulate around nuclei [2,24]. Differences in absorption frequency as a result of the group to which an atom is bounded can result to an effect, chemical shift. Chemical shift is important in the identification of functional groups and determination of structural arrangements of groups as different functional groups has different characteristics.

Spin spin splitting occurs when the magnetic moment of the electrons of a nucleus interacts with the magnetic moments of immediately adjacent nuclei. A change in electron distribution produces changes in the magnetic field of adjacent nuclei and causes splitting of energy levels and this causes multiple transitions. In spin spin splitting interactions the nuclear spins of adjacent atoms influences each other. Spin spin splitting permits the determination of how different functional groups are connected in a molecule since atoms of only adjacent functional groups can split each other.

In NMR spectra analysis, the following processes are involved.

(1) Comparison of signal chemical shifts with reference table to determine which functional groups may be present.
(2) Evaluation of the signal integrations to determine the number of equivalent H atoms represented by the signals.
(3) Construction of the possible molecules from the functional groups present, considering the relative signal integrations, the molecular formula or other information provided and any other spectra.
(4) Evaluation of signal splitting to ensure that proposed molecule is consistent with which functional groups that is adjacent to each other as observed by the splitting.

The major disadvantage of NMR is high cost, probability that resonance peaks will overlap as sample increases, low sensitivity and low identification power in relation to individual components of complicated mixtures.

3.2.2 Proton NMR

Proton NMR spectroscopy is important in identification and structural characterization of metal –ligands, organic and biochemical molecules such as protein, in the quantitative determination of absorbing species and in elemental analysis. NMR spectra are practically different from other spectra because there exist a direct proportionality between peak areas and the number of nuclei responsible for the peak. This property has made it possible not to use pure samples for calibration in the quantitative determination of a specific compound. NMR has been a valuable tool for the determination of functional groups in organic compounds such as hydroxyl groups in phenols and alcohols [2,24a].

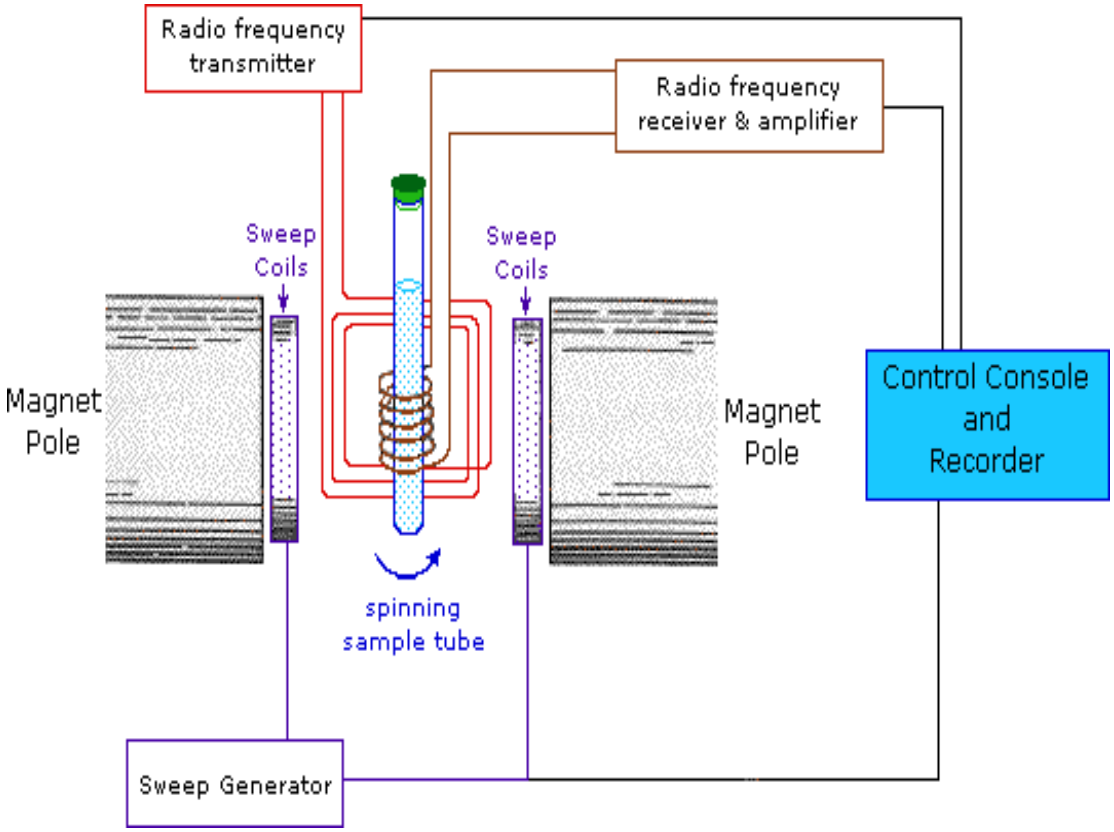

Fig. 3. Diagram of NMR (Adapted from [24b])

Carbon -13 NMR spectroscopy is advantageous compared to proton NMR spectroscopy in several ways; carbon-13 NMR provides information about the backbone of a given molecule rather than its periphery, it has a higher field strength magnets and Fourier transform instruments, it has higher chemical shift range of 200 ppm compared to 10 to 15ppm for the proton NMR, there is less overlap of peak and various methods of decoupling which results in the formation of one line spectrum by a particular type of carbon. The limitation of carbon-13 NMR is its low signal strength and small magnetogyric ratio which makes the method less sensitive than proton NMR. However, carbon-13 NMR is germane for structural elucidation which is dependent on chemical shifts with spin spin data less important as compared to proton NMR [2,24a].

3.3 Molecular Mass Spectroscopy

This is the most widely used of all the analytical tools since it provides scientists with valuable information about elemental components of samples of matter, gives the structures of inorganic, biological and organic molecules, gives qualitative and quantitative composition of complex mixtures, gives the structure and composition of solid surfaces and shows the isotopic ratios of atoms in the sample under investigation. The technique of molecular mass spectroscopy is superior to other spectral methods in the combination of features such as selectivity, generation of molecular mass/formula sensitivity, and combinability with chromate-graphy [26].

In molecular mass spectroscopy, the analyte which may be a vapour is always bombarded with a stream of electrons that leads to loss of electron by the analyte and consequent formation of molecular ion with the same molecular weight as the molecule. The collision imparts enough energy to the molecules making them to be in the excited state. Relaxations set in by fragmentation of part of the molecular ions to yield ions of lower masses. In the process, positive ions produced are attracted through the slit of mass spectrometers where they are characterized according to mass-to-charge ratio and displayed

in form of mass spectrum. The choice of instrument for identification of analyte depends on properties of analytes and data types necessary for characteristics identification [2]. These identification features include masses of the most important ions, masses of individual fragment ions and intensities of their mass peaks, accurate ion masses and corresponding molecular formulas and full mass spectrum. The properties of the analytes that are very important are polarity of the molecule, molecular mass and volatility.

There are various types of mass analyzers. These include quadrupole, triple quadrupole, ion trap, time of flight, quadrupole time of flight, orbitrap and ion cyclotron resonance [27]. The various mass spectrometers have different accuracies, and mass range with quadrupole time of flight having high mass range and accuracy high reproducible fragment spectra providing reliable identification [28]. The various types of mass analyzers are shown in Table 3.3.

According to guidelines on the use of mass spectrometry, for identification, confirmation and quantitative determination of residues [29], there are many methods of ionization or sources of ion in molecular mass spectrometry. They include electron ionization, chemical ionization, electrospray, atmospheric pressure chemical ionization, laser desorption / ionization and electron capture dissociation.

Different authors [2,24a,30] have noted that mass spectrometry is germane in the identification of molecular weight of compounds, the molecular formula, the functional group, and the actual identity of the compound can be established on comparison of the mass spectra with those of known compounds. One of the major limitations of molecular spectroscopy is its lower applicability in direct analysis of unpolar high molecular compounds.

3.4 X-ray Fluorescence Spectroscopy

X–ray fluorescence (XRF) involves the irradiation of the sample with a beam of x-rays from an x-ray tube or radioactive source leading to excitation of the element and consequently the characteristic fluorescence of the element is emitted. XRF has become a very common analytical method for qualitative identification of elements having atomic numbers greater than 8 (oxygen). XRF is most importantly used for quantitative or semi quantitative analyses of elements and in contrast to other analytical method is non destructive to the sample [31].

Studies [24a] have noted that x-ray fluorescence is important in the analysis of atmospheric pollutants in quantitative determination of elements heavier than sodium in rocks and soil, easily adapted to liquid samples and for quality control in the manufacture of metals and alloys. The spectra of XRF is relatively simple without spectral interference, non-destructive and as such can be used for the analysis of paintings, Jewelry, coins and archeological specimens without damage to the specimen. The procedure is also speedy (fast), convenient and allows multi-element analysis. The precision and accuracy of the method is higher than or equal to those of other analytical procedure. The major limitation of this method is that it is generally not as sensitive as most optical methods of analysis [32]. Apart from this, the method becomes more difficult in detection and measurement as the atomic numbers gets smaller than that of vanadium as the fluorescence intensity is reduced. Also the high cost of XRF instruments is a major limitation for its applicability.

3.5 X-ray Spectroscopy

X–ray spectroscopy is an analytical method based on the measurement of emission, scattering, fluorescence and absorption of electromagnetic radiation. The qualitative and quantitative determination of all elements in the periodic table having atomic numbers greater than that of sodium is the major application of x-ray absorption and x-ray fluorescence spectroscopy [24a].

X-rays are electromagnetic radiation of short wavelength generated by electronic transitions in the inner orbitals of atoms or by the deceleration of high energy electrons.

The emission of x-ray whose wavelength range is from $10^{-5}A^0$ to $100A^0$ can be achieved in four ways

(1) Bombardment of a target metal with a beam of high energy moving electrons.
(2) Use of radioactive source whose decay process yields x-ray emissions
(3) Exposure of a substance to primary beam of x-ray to generate a secondary beam of x-ray fluorescence
(4) From a synchrotron radiation which has limited usage because of its unavailability.

X-ray diffraction is most importantly employed in the analysis of crystalline materials. Currently, x-ray diffraction is important in the structural elucidation of complex materials and in the qualitative and quantitative analysis of solid samples. X-ray diffraction exploits structural elucidation of inorganic and organic solids and identification of crystalline phases by means of diffraction theory and or comparison of intensities and positions of the diffraction peaks to libraries of established crystalline substances [2].

The major advantages of x-ray diffraction are

(1) Other modern spectroscopic method of analysis mainly focuses on characterization of the synthesized target at a molecular level within a short time whereas x-ray diffraction gives detailed information on the molecular assembly in the solid state.
(2) X-ray diffraction provides direct information on the arrangement of molecules in the organic solid state.
(3) The emergence of x-ray powder diffracton (XRPD) has provided an alternative to single crystal x-ray diffraction which focuses only on structural information. XRPD is now used for characterizing solid state supramolecular architectures (crystal structures) [33].

3.6 UV-vis Spectroscopy

Ultraviolet / visible molecular absorption spectroscopy is an analytical technique based upon electromagnetic radiation in the wavelength region between 160 to 789 mm. The method is useful for the quantitative determination of a large variety of inorganic and organic species [2,24]. Molecular absorption spectroscopy is based on the measurement of the absorbance A or transmittance T of solutions in transparent cells with path length b (cm). The relationship between the concentration of the analyte and the absorbance of the solution is linear as shown by Beer Lambert's law.

$A = \log T = Ebc$

A = Absorbance, T = transmittance, E = Molar absorptivity
C= Concentration and b = path length

The major application of UV-visible spectroscopy is in assaying of analytes although it can also be used in identification and determination of metal ligand mole ratio in a complex which invariably helps in structural identification. However, its limitation lies on interferences and instrumental limitations.

3.7 Molecular Fluorescence

Molecular fluorescence involves the excitation of an analyte to give species whose emission spectrum gives information useful in qualitative and quantitative analysis. Fluorometers and spectro flurometers are instrument for measuring the fluorescence of substances [24a]. Fluorometric methods are used for the direct determination of inorganic species by formation of a fluorescing chelate and measurement of its emission. This is similar to spectrophotometry where a chelating agent complexes with a metal ion prior to its determination [34-37].

3.8 Electron Spins Resonance Spectroscopy (ESR)

Electron spin resonance spectroscopy is an analytical technique used for studying metal complexes, inorganic radicals or organic radicals and biomolecules. Electron spin resonance also regarded as electron paramagnetic resonances is based on the fact that an electron is a charged particle which spins around its axis which invariably causes it to behave like a magnet. Electron spin resonance spectroscopy is a very powerful and sensitive method of characterizing electronic structures of materials with unpaired electrons [38].

Electron spin resonance is a technique based on the interaction of unpaired electron spins with an external magnetic field. In ESR, the electrons in the lower energy level of the spectrometer magnet are excited to the upper energy level while the sample is exposed to fixed microwave irradiation. A modern form of ESR/EPR is ENDOR (electron nuclear double resonance) which has high resolution and capable of measuring nuclear magnetic transition frequencies in paramagnetic systems. The important application of ENDOR is that the NMR spectrum of nuclei which interacts with a paramagnetic center is measured.

Although ENDOR is a very powerful tool the limitations include the need for advanced equipment which is costly and generation of sufficiently high radio frequency power as well as the coil to generate the appropriate magnetic field which is often a hindrance [38]. Also important to note is that ENDOR technique needs low temperature for measurement and as well a sensitive ESR spectrometer.

Table 3.3. Modern mass analyzers and their combinations

s/n	Mass analyzer	Mass range	Mass accuracy	Fragments for identification
1	Quadrupole	+	+	E1 from ++ to +++ ES1:+
2	Triple quadrupole	+	+	ES1:++
3	Ion trap	+	+	ES1: to ++
4	Time of flight	+++	Up to +++	MALDI:+
5	Quadrupole time of fight	From + to +++	Up to +++	ES1 and MALDI:++
6	Orbitrap	+	+++	From + to ++
7	Ion cyclotron resonance	+	+++	From + to ++

+ = low, ++ = medium, +++= high for mass range and accuracy.EI= Electron ionization, ESI = Electrospray ionization; MALDI= matrix assisted laser desorption /ionization

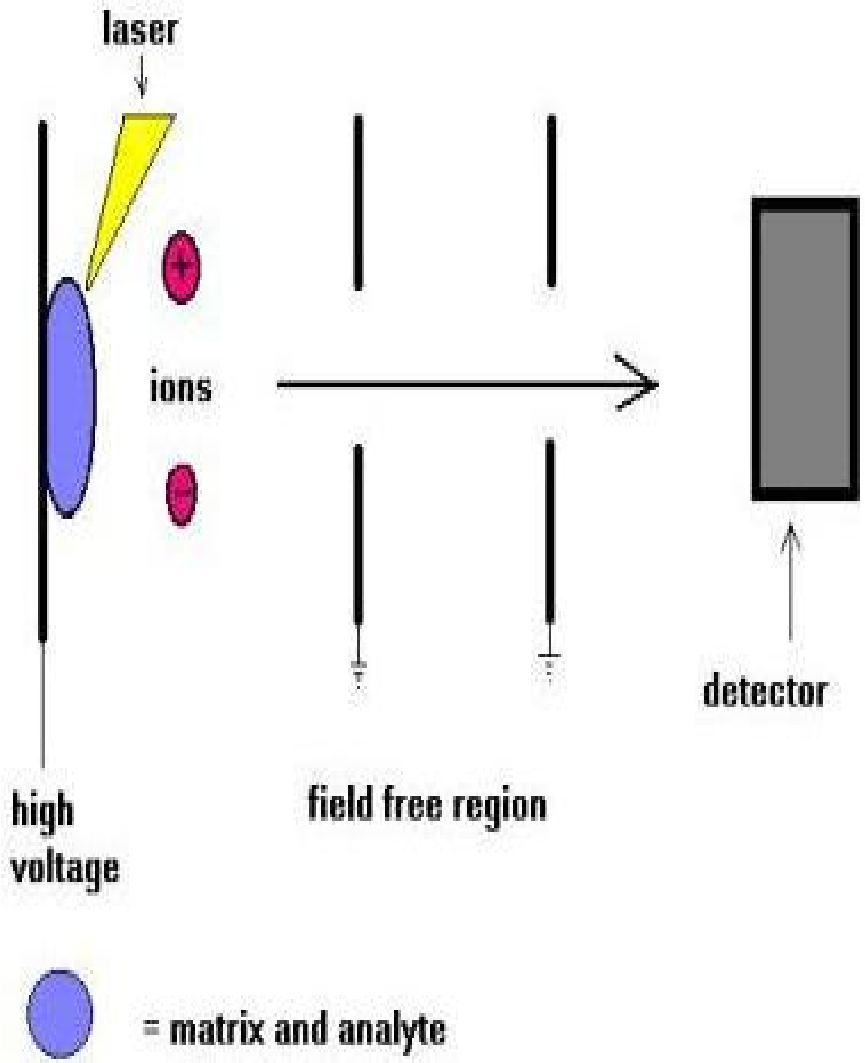

Fig. 4. Diagram of Matrix Assisted Laser Desorption (MALDI) [Adapted from [24b])

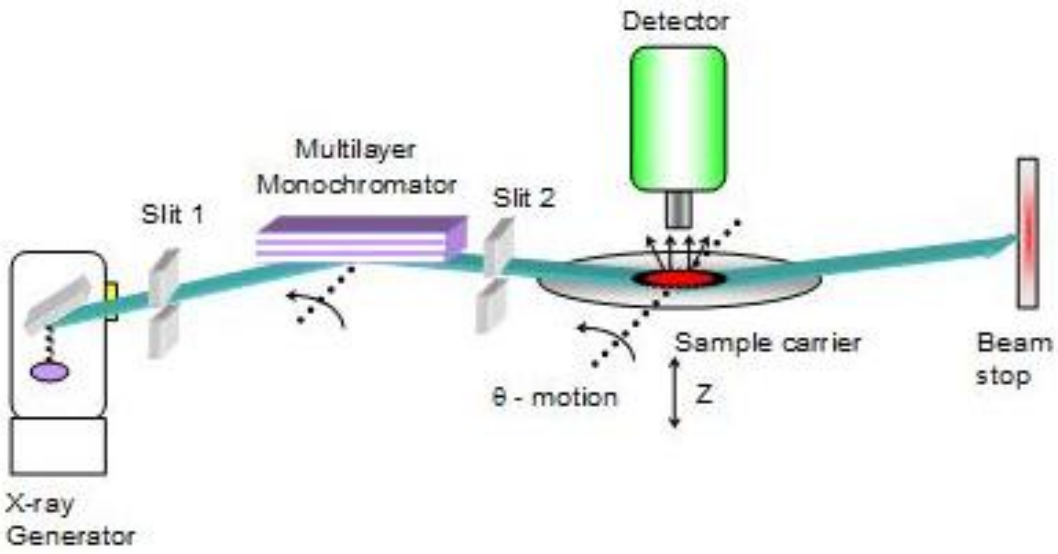

Fig. 5. Diagram of XRF spectrometer (Adapted from [24b])

Table 3.4. Comparison of some Techniques of molecular spectrometry (-scopy) for identification

Technique	Principle	Main applications	Limitations
UV-vis	Measurements of light absorption at different wavelengths in ultraviolet (wavelengths in ultraviolet (wavelength 190-400 nm) and visible (wavelengths 400-780 nm) part of the spectrum due to electronic excitation	Detection for HPLC	Spectra characterize chromophore types rather than individual compounds
IR	Absorption measurement of IR radiation (wavenumbers from 13,000 to 10 cm^{-1}, wavelengths from 0.78 to 1,00 μm) due to vibration excitation	Structure elucidation (determination of functional groups) qualitative analysis (polymers, plastics, resins, food, and so on)	Relatively low sensitivity (≥-10 μg is commonly needed for spectral recording); low compatibility of IR detector with GC and especially LC
NMR	Absorption of radiation in the radiofrequency range of the electromagnetic spectrum (hundreds of MHz) due to changes in the spin states of the atom nucleus	Structure elucidation of pure compounds, metabolomics qualitative analysis	Relatively low sensitivity (≥ 100 ug is commonly needed for ^{1}H spectral recording, with lesser amounts in a few hours acquisition time); slow progress in LC-NMR
MS	Measurement of mass (up to 10^{6} Da) and amount of ions (down to a few counts) generated from atoms/ molecules of a substance	All kinds of chemical analysis	Lower applicability in direct analysis of un polar high molecular compounds

Table 3.5. Comparison of different analytical techniques

s/n	Technique	Acronym	Description	Minimum sample size	Sample preparation	Sensitivity	Specificity
1	Polarized light microscopy	PLM	Identification of material based on physical properties	5 µm	Easy	n/a	None
2	Infrared spectroscopy	IR	Compositional analysis of organic and inorganic compounds	10 µg	Easy	10%	None
3	X-ray fluorescence	XRF	Elemental analysis	Non-destructive 1 mm spot	None	0.1%	Elements only (heavier than potassium)
4	Energy dispersive spectroscopy	EDs	Elemental analysis similar to XRF but attached to scanning electron microscopy	1 µm spot	Easy	0.1%	Elements only (heavier than carbon)
5	X-ray diffraction	XRD	Compositional analysis of crystalline material	10 µg	Easy	5%	Crystalline materials
6	Inductively coupled plasma	ICP	Quantitative/qualitative analysis of elements	1 mg	Slow	0.01 ppm	Elements only (heavier than nitrogen)
7	Chromatography high performance liquid	HPLC	Quantitative/qualitative analysis of organic components in a mixture	1 µg	Slow	0.01 ppm	General class of materials in mixture must be known before measurement of analysis
	Gas	GC		1 µg	Slow	0.01 ppm	
	Thin layer	TLC		1 mg	Slow	5%	

4. CONCLUSION

Spectroscopic methods of analysis and diffraction methods of analysis are complementary techniques or sometimes the former being an alternative to the traditional diffraction method. The determination of structure of complexes is a factor influenced by the periodicity of the structure whereas spectroscopic method provides valuable information about the structure of the complex (chemical and crystallographic environment), co-ordination number, site, symmetry but independent of long range periodicity or crystallinity as diffraction methods. Diffraction methods involve a change in direction of the incident radiation without change in energy. All spectroscopic methods involve the same basic principle of absorption, transmission, reflection, scattering or emission of incident beam of radiation by matter/from material.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Thieme D, Muller RK. Information theory and systematic lexicological analysis in "general unknown" Poisoning cases. Fresenius J Anal Chem. 1997;358: 785-792.
2. Milman BL. Chemical identificaiton and its quality assurance. Springer –verlag Berlin H.2011,23-39.
3. Eckschlager K, Danzer K, Matherny M. Informution slheorie in der Analytik. II: Mehrkomponenlenanalyse. Fresenius Z Anal Chem. 1989;334:1-8.
4. Rafltery D. High-throughput NMR spectroscopy. Anal Bioanal Chem. 2004; 378:1403-1404.
5. Hornak JP. The basics of NMR; 2002. Available:http://www.cis.rit.edu/hbooks/mmr (Accessed 30th October, 2014)
6. Hsu CPS. Infrared spectroscopy. in: Settle FA (ed) Handbook of Instrumental Techniques for Analytical Chemistry. Prentice Hall; 1997. Available:http://www\\.jircnhall.com/sclIle/cl uipiers/chl5-.pdf. (Accessed 30th October, 2014)
7. Ebsworth EAV, Rankin DWH, Cradock S. Structural methods in inorganic chemistry. 2nd Edition Blackwell Scientific Publications Boston. 1991;25.
8. Croates J. Interpretation of infrared spectra, a practical approach. in: Meyers RA (ed)/ Encyclopedia of Analytical Chemistry. Wiley, Chichester. 2000;10.
9. Ferraro JR, Nakamoto K, Brown CN. Introductory Raman spectroscopy. Academic Press. Sandiego C A; 2003.
10. Ladd MFC, Palmer RA. Structure determination by X-ray Crystallography. Kluwer, New York. 2003;14
11. Blow DM. Outline of crystallography for biologister. Oxford University Press, Oxford; 2002.
12. Mavridis IM. Structural characterization of inorganic biomaterials. In Biomedical Inorganic Polymers, Progress in Molecular and Subcellular Biology. 2013;54:19-58.
13. Kapoor S, Batra U Preparation and bioactivity evaluation of bore like hydroxyapatite –Bioglass composite. Int. J. Chem Bio Engin. 2010;13:24-28.
14. Cole RB. Electrospray ionization mass spectrometry. Fundamentals, Instrumentation and Applications. Wiley Interscience, New York. 1997;25.
15. Traeger JC, Cotton R. Advances in mass spectrometry. Proceeding of the 14th International Mass Spectrometry Conference. Tampere Finland 25-29, August; 1997.
16. Henderson W, Scott Mcindoe H. Electrospray mass. Spectrometry Comprehensive Co-ordination Chemistry II. 2003;2:387-391.
17. Hunsucker SW. Time of flight mass spectrometry to characterize inorganic co-ordination complexes and cyanobacteria. Dissertation. Presented to Department of Chemistry, Virginia Polytechnic Institute and State University. 2001;1-7.
18. Pessoa JC, Coneia I, Goncalves G, Tomaz I. Circular dichroism in co-ordianton compounds. Journal of the Argentine Chemical Society. 2009;97:151-165.
19. Boas VL, Pessoa CJ. Vanadium in comprehensive co-ordination chemistry. Ed. Wilkinson G, Gillard RD and McCleverty JA. Oxford. 1997;3:453-583.
20. Chu B. Laser light scattering. 2nd Edition Academic Press, New York. 1991:30.
21. Mohammed GG, Omar MMM, Hindy AH. Metal complexes of schiff bases. Preparation characterization and biological activity. Turk. J. Chem. 2006;30:361-382.
22. Dean JA. The analytical chemistry handbook. New York, McGraw Hill 2nd. Editon. 1995;60:21.

23. Hammouda HOD, Kline BS, Chen WR. Unusual phase behaviour in mixtures of polyethylene oxided and ethyl alcohol. J. Polymer Science. Polym. Phy. Ed. 2006; 44:557-564.

24a Skoog DA, Holler JF, Nieman TA. Principles of instrumental analysis. Fifth Edition. Hencourt Brace College Publishers USA. 1998;3:1-5.

24b Available:Chemwiki.ucdavis.edu (Accessed 30th July, 2015)

25. Michelle RD, Dusan S, James ML. Infrared spectroscopy in conservation science. 1st Edition J. Paul Getly Trust, USA. 1999; 1-252.

26. Milman BL, Konopclko LA. Modern mass spectrometry: Proportions of develop-ments. Mass Spectrom (Moscow, in Russian). 2006;3:271-276.

27. Schuhmacher R, Sulyok M, Krska R. Recent developments in the application of liquid chromatography-tandem mass spectrometry for the determination of organic residues and contaminants. Anal Bioanal Chem. 2008;390:253-256.

28. Schaeffer-Reiss C A. Brief summary of the different types of mass spectrometers used in proteomics. In: Thompson JD, Schaeffer-Reiss C, Ueffing M (eds) Functional proteomics: Methods and Protocols. Humana Press, Totowa, NJ; 2009.
Available:http://www.springeriink.corn/conlent/43522nl40137640 (Accessed 30th October, 2014)

29. Commission Decision. Implementing council directive 96/23/EC concerning the performance of analytical methods and interpretation of results off. J Eur Commun L. 2002;221:8-36.

30. Currie LA. Nomenclature in evaluation of analytical methods, including detection and quantification capabilities (1UPAC recommendations). Pure Appl Chem. 1995; 67:1699-1723.

31. Rios A, Escarpa A, Gonzalez MA, Crevillen AG. Challenges of analytical microsystems. Trends Anal Chem. 2006; 25:46779.

32. Farre M, Brix R, Barcelo D. Screening water for pollutants using biological techniques under European Union Funding during the last 10 years. Trends Anal Chem. 2005;24:532-545.

33. Shyam K, Laszlo F, Tomislav F, William J. Powder x-ray diffraction as an emerging method to structural characteristic of organic solids. Organic Letters. 2007;16: 3133-3136.

34. Filipiak M. Electrochemical analysis of polyphenolic Compounds. Anal Sci Suppl. 2001;17:M667-JI670.

35. Lund W. Speciation analysis why and how? Fresenius J Anal Chem. 1990;337: 557-564.

36. Nworie FS, Nwabue FI. Solvent extraction studies of metal complexes derived from a tetradentate Schiff base Bis(Salicylidene) ethylenediamine (H_2SAL) in Acid Medium. Int. Journal of Innovative and Research. 2014;2:66-75.

37. Ukoha P, Obasi L, Nwabue FI. Solvent extraction studies on copper (II) and silver (I) complexes of Bis(4-hydroxypent-2-ylidene)diaminoethane: Composition of extracted species. E-Journal of Chemistry. 2012;8:1864-1871.

38. Weckhuysen BM, Heidler R, Schoonheydt RA. Electron spin resonance spectroscopy. Mol. Sieves. 2004;4:295-335.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/11194